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ISOLATION, CHARACTERIZATION AND EXPRESSION ANALYSIS OF PUTATIVE DROUGHT RESPONSIVE EXPRESSED SEQUENCED TAGS FROM *GOSSYPIUM ARBOREUM* ROOTS

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ABSTRACT

Cotton is an important economic fibre crop. Seasonal water shortages and long term water deficit can effect cotton yield. Current study aimed to explore the cotton root transcriptome under drought stress. mRNA extracted from cotton roots subjected to osmotic stress treatment (5% gravimetric humidity) was used to construct cDNA library. Expressed sequence tags submitted to gene bank EST database (JK757087-JK757798) further annotated to predict the homology and function. Total 104 transcripts with an E-value less than $1e^{-33}$ revealed 82 (78.84%) known homologs and 22 (21.15%) with uncharacterized proteins. Gene ontology and KEGG analysis of these drought responsive ESTs elucidated their key role in biological regulation, molecular functions and cellular organelles. Expression pattern of 10 unigenes were validated by RT-qPCR in roots and leaves. These unigenes included WD repeat, FRIGIDA, peroxidase, E3 ubiquitin ligase, U-box domain, RNA binding, calcyclin binding, Glutathione S transferase, endochitinase and metallothionine like protein. cDNA library was successfully constructed from cotton roots and revealed several key drought responsive transcripts. Novel sequences identified in this study can be valuable resource for further exploration studies to exploit their role in genomics of drought responsive mechanism.

Key words: Drought stress; Expressed Sequence Tags (EST), Homology, KEGG, Orthologs, qRT-PCR.
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INTRODUCTION

Cotton is an important economic fibre crop, grown in tropical zones especially in U.S, Uzbekistan, China, India, Brazil, Pakistan and Turkey (Riaz *et al*., 2013). As stated by measurable surveys, India, USA, China, Brazil and Pakistan are top five principal cotton producing countries in the world (Statista 2019; OECD-FAO 2019). Cotton production declined in India, China, USA and Pakistan mainly due to water shortage and pest problems (OECD-FAO 2019). Unfavourable environmental abiotic stresses are leading factors in decreasing agricultural productivity (Grayson, 2013). Indeed, cotton being as glycophyte shows higher degree tolerance to abiotic stresses. However, extreme natural factors like drought affect growth, productivity and as well fibre quality of cotton (Parida *et al*., 2007). The molecular biology approaches play key role in genome alteration of higher plants against environmental factors for better growth and yield (Edgerton, 2009; Lawlor 2013). Incapacitating abiotic factors that reduce the crops yield has been main area of discussion. Drought, key abiotic factor that significantly affects plant biomass and yield (Chaves and Oliveira, 2004). The cotton genome is large as compared to other plant species, making it challenging to study. Thus, key understanding of drought stress tolerance can disclose the variable expression and regulation of key genes that may augment cotton drought tolerance.

Drought includes a variety of plant responses, including stomatal regulation, gene expression alteration, build-up of abscisic acid, generation of osmotic compounds and production of defensin proteins that destroy free radicals, ROS or act as nucleic acid binding factors (Wang *et al*., 2003). These events are controlled by complex networks at molecular level that trigger stress responsive mechanism to restore homeostasis, defend and revive impaired cellular components (Ramachandra *et al*., 2004). Responses to abiotic factors are genetically intricate and also complicated to understand. Previously, gene expression encoding dehydrins, antioxidants involved in the generation of structural and functional metabolites were used for modification of stress tolerance in plants (Park *et al*., 2005). Currently, approaches to utilize genes with their role in signalling and monitoring

networks and pathways have revealed remarkable potential (Umezawa *et al*., 2006).

Previous studies have focused on the cotton drought resistance on aboveground plant tissues while less knowledge have been documented on underground plant tissues. For instance, 3,517 unigenes were differentially expressed in *Gossypium herbaceum* leaves and roots of cotton with involvement of the 28 biological pathways significantly to drought stress (Ranjan and Sawant, 2015); cDNA library analysis of the 92 positive drought stress responsive clones with differential expression (Zhang *et al*., 2009); 6,047 high-quality expressed sequence tags (ESTs) from *G. barbadense* revealed enrichment of transcription factors and stress-related genes (Zhou *et al*., 2016).

Roots are the vital organs of plants with key role in absorption and translocation of nutrients and water. Being as major connection between the plant and soil stresses, root generate specific chemical messenger from root to shoot that initiate stomatal closure and ultimately reduce evaporation losses (Davies and Zhang, 1991; Jia and Zhang, 2008). However, limited information prevails at molecular level regarding cotton root responses under water deficit stress (Graya and Brady, 2016). A key understanding of principal genes involved in osmotic stress is necessary for the plant development that sustain more yields under osmotic stress.

Sequenced and re-sequenced cotton genomes are simply the foundation; the main challenge is to discover the features of the genome to elucidate the biology. The next stage of cotton genomics will entirely expose these biologically genome active states, as has been made for other model crop plants where high density genetic and fine maps, SNP array platforms, transcript abundance epigenetic regulations and modifications (Ashraf *et al*., 2018). Recently, most promising molecular approach is transcriptome profiling for demonstrating how information obtained from sequence data can be transformed into an extensive knowledge of gene function. Genome sequence and latest approaches like NGS technology practiced and reported several reports in cotton using RNA-Seq analysis. For instance, cotton root transcriptome analysis under water deficit stress (Bowman *et al*., 2013; Zhang *et al*., 2016) has been reported. However, RNA-Seq technique being among latest molecular approaches to study gene annotation faces some challenges such as library construction hence screening cDNA libraries screening as high throughput approach is an efficient way to identify functional and stress-tolerance genes in cotton (Li *et al*., 2019) and other plant species (Wang *et al*., 2019; Dossa *et al*., 2019).

Despite of being the massive cotton genome sequence information using latest genomic approaches like whole genome sequencing and re-sequencing, still there large information gaps as compared to other model plants like tobacco and Arabidopsis. Hence, next era of cotton genomics require re-sequencing broad diversity panels, draft genome refinement including the development of high throughput functional genomics tools and integrating multidisciplinary approaches including transcriptomics, epigenomics, proteomics and bioinformatics to further explicate its genome and functional characterization.

Our lab has previously reported the abiotic stress responsive genes in *Gossypium arboreum* using multiple molecular approaches and tools (Maqbool *et al*., 2008; Barozai and Husnain 2012; Shahid *et al*.,2012). Previous findings revealed the elucidative role under multiple abiotic stresses. Taking into account the Gossypium arboreum as potential gene pool of abiotic stress responsive genes, present study was planned to explore the key putative drought responsive transcripts in cotton root by cDNA library. In this study, we report the identification and functional characterization of young root drought responsive based EST's of cotton.

MATERIALS AND METHODS

Plant growth, drought stress induction, RNA isolation and mRNA purification: *Gossypium arboreum* cv FDH-786 was selected for evaluation. Delinted seeds were grown in a mixture of peat, sand, soil (1:1:1) under controlled environmental conditions in green house at 25±2°C; relative humidity 45-50% and 1500 $\mu molm^2s^{-1}$ light intensity provided by metal halide lamps (400 W). The water stress treatment was inducted following previous studies (Maqbool *et al*., 2007; Jamal *et al*., 2014). The amount of water held by the soil was measured as gravimetric humidity (GH). Forty days old cotton seedlings following two moisture stress treatments 10% and 5% GH along 15% GH as control treatment were taken into study. Water stress treatment was maintained periodically for 15 days and monitored gravimetrically by weighing the pots daily. The fresh roots and leaves were harvested, immediately frozen and grinded in liquid nitrogen for RNA isolation. To construct the drought responsive cDNA library, RNA extraction was done form plants maintained at 5% GH.

The relative water content (RWC) of leaves were measured for the second fully expanded leaves. The RWC was measured as described earlier (Barrs and Weatherly, 1962).

RWC (%) = [(fresh wt – dry wt) / (turgid wt – dry wt)] × 100

Total RNA isolation from the roots and leaves was performed as described earlier with minor modifications (Jakola *et al*., 2001). Isolated RNA was further treated with DNase I, RNase-free (Thermo Fisher Scientific, USA) to avoid genomic DNA impurity before the synthesis of mRNA. To check the integrity of RNA samples, RNA samples were electrophoresed on 0.9% agarose. RNA concentration was measured using

spectrophotometer (ND-1000 NanoDrop Technologies, Inc.). RNA samples having A260/280 ratio of 1.8-2.0 were used further. mRNA extraction and purification was performed using oligotex mRNA mini kit (Qiagen, Valencia USA) following manufacturer directions.

cDNA library construction, clones amplification, Sequencing and bioinformatics: cDNA library was constructed using CloneMiner™ cDNA library construction kit (USA, Invitrogen) following manufacturer instructions. Blunt end ds cDNA was size fractioned using low melt agarose gel (0.8%) with size ranged between > 100 bp - <1 kb. The ds cDNA was eluted using DNA gel extraction kit (Thermo Fisher Scientific, USA) following manufacturer guidelines. The eluted cDNA was proceeded for BP recombination reaction. Electroporation was performed for fractioned cDNA aliquot by adding to thawed ElectroMAX DH10B TM T_1 phage competent resistant cells. Electroporated cells were incubated at 37ºC for 1 h at 200 rpm for the expression of kanamycin. Incubated cells were further pooled with equal volume of sterile freezing media. These aliquots were prepared from pooled samples and stored at -80ºC. Clones were screened from white colonies on agar plates with kanamycin selection. Positive transformants were confirmed by colony PCR. Colony PCR was performed using M13 sense and antisense primers following amplification program of initial denaturation at 94ºC for 5 min; denaturation at 94ºC, annealing at 52ºC and extension at 72ºC each of 35 cycles at 45 s, 45 s, 60 s respectively and final extension at 72ºC for 10 min.

For sequencing, isolated plasmid DNAs from randomly selected clones were used for Sanger sequencing. Isolated plasmids were extracted using alkaline lysis method (Sambrook *et al*., 1998).Clones were sequenced using cycle sequencing kit (ABI PRISM Foster, USA) on Applied Biosystems Sequencer model 3100/3700. The vector and adaptor sequences present at both 5′ and 3′ of sequences were removed using Vec Screen online available tool (https://www.ncbi.nlm.nih.gov/tools/vecscreen/). NCBI database was used to study the non-redundant nucleotide sequence (BASTN) and non-redundant protein sequence (BLASTX) similarities between ESTs and other databases sequences. Unisequences with an E-value < $1e^{-33}$ were compared to swissprot and blastx (Altschul *et al*., 1990). Analyzed blastx predicted sequence homology against Gossypium species were further used in Cotton Functional Genomics Database (CottonFGD) (https://cottonfgd.org/) to find the Gene ID of the respective sequences. The gene ids of the respective sequences saved were used for further annotation and functional assignments. The gene annotation (functions) of cotton was performed using PANTHER (ver 13) (http://pantherdb.org/) using corresponding Arabidopsis orthologs. KEGG Mapper (Kyoto Encyclopedia of Genes and Genomes) (Ogata *et al*., 1999) (http://www.genome.jp/kegg/mapper.html) was used to study the KEGG orthology and associated pathways online available.

Quantitative real time PCR analysis (RT-qPCR): Total RNA was treated with DNase I to avoid any genomic DNA residues. First strand cDNA was synthesized using cDNA synthesis kit (Fermentas, Germany). Primers were designed against using online available tool (http://bioinfo.ut.ee/primer3-0.4.0/) (Table 1) with selection of no primer dimer synthesis. Cotton GAPDH primer was used as reference gene for normalization (Zahur *et al*., 2012). Quantitative real time PCR was performed using iQ5 (Bio-Rad, USA) with IQTM SYBR Green supermix (Fermentas, USA). Amplification program consisted of 95 ºC for 3 min, then 40 cycles of amplification at 95ºC for 30 s, 60ºC for 30 s, 72ºC for 30 s. Melting curves were obtained from 70ºC to 95ºC at 0.1ºC/s by continuous monitoring of fluorescent signals to check the specificity of amplicons and primers dimers. Each reaction was set in triplicate for both technical and biological to minimize any variation. Reactions were set in volume of 20 µL containing 200 ng cDNA, 15 µL 2X SYBR Green supermix and 1.0 µL (10 µM) of each primer. CT (cycle threshold) values were analyzed later using iQ5 software (Bio-Rad Ver 1.0). The cycle threshold (CT) values were exported to MS Excel for further analysis. To study the relative gene expression level, comparative ct method was adapted. The CT values were normalized with internal standard and the fold differences were calculated using delta approach (Livak and Schmittgen, 2001).

RESULTS

Physiological performance of cotton under drought: RWC from cotton leaves were found to be 70.23%, 55.12% and 44.30% with plants grown at 15%, 10% and 5% GH (gravimetric humidity) levels respectively. A pronounced reduction in RWC of leaves was measured at 5% GH level in comparison to other treatment.

Construction of cDNA library and functional characterization of cDNA sequences: Total 800 clones were randomly selected for sequencing. After sequencing, 711 clones showed an insert size of 100-800 bp. Total 711 unigenes (Accession# JK757087-JK757798) were submitted to NCBI Genebank. NCBI BLASTN revealed that 76% (541 sequences) showed sequence similarity to known sequences. Majority of blast match hits belonged to *Gossypium spp, Populus trichochorpa spp, Oryza sativa, Zea Mays, Glycine max, Medicago, Nicotiana spp, A. thaliana* and *Ricinus spp, Atriplex* and other plant species. Similar ESTs were simultaneously annotated for their protein functions to

categorize into their classes. The BLASTX results demonstrated 104 unigenes showing significant similarity to known genes, 82 uniESTs displaying significant similarity to genes of predicted proteins, and 22 uniESTs remain uncharacterized in NCBI database.

The gene ontology terms were further used to classify the gene products with an E value $1e^{-33}$ in functional GO categories and simplified into plant-specific annotations (GO classification) to obtain additional insights into the putative functions of unigenes. Of the 104 *G. arboreum* ESTs, 82 (78.84%) were assigned GO terms in any category (biological, cellular and molecular), and the other 22 (21.15%) ESTs were uncharacterized proteins without GO terms annotations. EST's which had no Arabidopis homology in NCBI genebank, failed to obtain a GO term, fell into distinct categories like uncharacterized, predicted and hypothetical proteins. We identified 82 unigenes from the 711 total ESTs, representing non-redundant unigenes, that share similarities with defense realted genes and stress response according to GO classifications. These 82 unigenes with Arabidopis based known homologs were further annotated to molecular function 30 (83%), 62 (100%) to biological process and 44 (100%) to cellular components. Many of the EST's in molecular function category (53%) were associated with catalytic activity followed by binding activity (30%), whereas the remaining ESTs were involved in structural molecule, translation and transport activity (Fig 1A). Within the category of biological process, 23 EST's (37%) were relegated to metabolic process, 19 (30%) to cellular process while others assigned to response to stimulus, biogenesis, localization and regulation (Figure 1B). Based on the cellular components, 20 (45%) EST's contributed maximum to cell part and 10 (22.7%) to macromolecule complex followed by organelle, membrane and cell junction (Figure 1C). EST's were also grouped based on the protein categories such as nucleic acid binding & hydrolase (19.6% each), enzyme modulator (10.7%) followed by chaperone, transferase, isomerase, lyase, cytoskeletal, oxidoreductase, signalling, storage, membrane trafficking, carrier, transporter, cell adhesion and calcium binding protein (Figure 1D).

BLASTX results showed significant drought responsive genes (Table 2). Some of these genes revealed sequence homology with transcription factor JUNGBRUNNEN 1, WD repeat, heat shock, FRIGIDA, peroxidase P7, glutathione S-transferase, potassium channel KAT1, ubiquitin carboxyl-terminal hydrolase, U-box domain, serine/threonine kinase, polyubiquitin, Zinc finger, lysine histidine transporter, junction-mediating and regulatory, 26S protease regulatory subunit, endochitinase, metallothionein, translational activator, CBL-interacting protein, ubiquitin, calcyclin-binding, RNA-binding like proteins isoforms. The detailed descriptions of 104 unigenes was obtained by BLASTX (Table 2).

Predicted KEGG pathways and validation of differentially expressed selected unigenes: The predicted KEGG pathways included HSP20 family protein, phosphatidylinositol glycan, large subunit ribosomal protein L23e, chromodomain-helicase-DNA-binding protein, DNA polymerase delta subunit, peroxidase, DNA-3-methyladenine glycosylase II, Peptidyl-prolyl cis-trans isomerase A, phosphoglucomutase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, translation initiation factor 1A, Ubiquitin, 1,3-beta-glucan synthase, crossover junction endonuclease EME1, 26S proteasome regulatory subunit T1, NADH dehydrogenase, carbonic anhydrase, proline iminopeptidase, calcyclin binding protein, auxin influx carrier, protein O-GlcNAc transferase, histone H2A, small subunit ribosomal protein S8e, gibberellin 2-oxidase, U3 small nucleolar RNA-associated protein, cell division protease, calmodulin and glutathione S-transferase (Table 3). This ultimately proves and evidence that the potential drought responsive unigenes in our study showing the BLASTX results strongly support their direct and indirect involvement in different pathways with similar role (Table 2, Table 3).

We selected ten ESTs with their known function in response to stresses: WD repeat (*WD*, JK757101), FRIGIDA (*FRIGIDA*, JK757130), Peroxidase P7-like isoform (*POX*, JK757160), U-box domain (*U-box*, JK757206), E3 ubiquitin-protein ligase (*E3LIG*, JK757286), RNA-binding (*RBP*, JK757358), calcyclin-binding (*CBP*, JK757361), glutathione S-transferase (*GST*, JK757362), endochitinase (ECHT, JK757585), metallothionein (*MTT*, JK757720) like proteins to further validate the expression of these genes in response to drought stress in cotton roots. The real time PCR showed significantly elevation of all 10 selected unigenes in root tissues as compared to leaf tissues. Among the 10 unigenes, *WD* (JK757101), was most up-regulated (223.8 fold) followed by *FRIGIDA* (188.40 fold), *POX* (100.22 fold), *U-Box* (72 fold), *E3LIG* (63.13 fold), RBP (53.46 fold), *CBP* (41.86 fold), *GST* (31.65 fold), *ECHT* (7.89 fold) and *MTT* (6.64 fold) at 5 % GH. Variable expression was measured with similar sequential pattern under 10% GH (Fig. 2). In leaves, subjected to drought stress at 5% GH, the expression pattern was significantly higher than 10% GH but it was significantly less than that of expression pattern observed in root tissues. *WD* (JK757101) showed the maximum up-regulation (51.73 fold), followed by *U-Box* (31.90), *POX* and *FRIGIDA* with similar expression level, *CBP* and *E3LIG* with similar expression pattern, *RBP* (18.40 fold), *GST* (15.81 fold), *MTT* (6.79) and *ECHT* (5.09) (Fig 2).

Table 1. Sequences of qRT-PCR primers used in this study.

Gene	Accession no	Forward primer (5′-3′)	Reverse primer (5′-3′)
WD	JK757101	TTTGTTGGGGTTGCTGATCG	CAAGGGCAAAACTAAACCTGC
FRIGIDA	JK757130	AGAAGCAGCCACTCACCTAG	AACACACAGGCATTGCTACC
POX	JK757160	TTCCTGCACCAACTTCGAAC	AATTGTTGTCCCCTGAGCCT
U-BOX	JK757206	AATTCTGTGCCGACAATGGG	CCAGCTTCAATCAGACAAGACC
E3LIG	JK757286	TCTCCATGTTGCCACCATCT	CAACACTACACTTGTACGCACT
RBP	JK757358	GCACTTGAGTCTGGTTGCAA	TTGGCGTGGTATCTCTCTCC
CBP	JK757361	CCTCCTTCAGCTGGGATCAA	TCACCTTGCACTTCTCTGGT
GST	JK757362	GGGCAGGCTTTGGTTAATGA	ACGAAAGATTCCCGACCGAA
ECHT	JK757585	GCTACTGGTTTCCTGGACGA	ATGGCTTTGATGGTTGCTCC
MTT	JK757720	AAACCATCCCCTCCCTTCTC	TTCAGCTCCATCAAAGTGCG

Table 2. Homology analysis of the transcripts with E-value < $1e^{-33}$.

Sequence ID	Length (bp)	Homology (blastx)	Species	Accession no	E-value
JK757090	568	Transcription factor JUNGBRUNNEN 1-like	*Gossypium arboretum*	XP_017614396.1	7e-66
JK757101	426	WD repeat-containing protein like isoform	*G. arboreum*	XP_017621565.1	3e-15
JK757105	404	X1	*G. arboreum*	KHF98356.1	6e-40
		Endochitinase			
JK757112	333	Uncharacterized protein LOC107907231	*G. hirsutum*	XP_016689998.1	3e-7
JK757113	731	Heat shock protein, mitochondrial-like	*G. hirsutum*	XP_016721162.1	6e-93
JK757118	439	isoform X2	*G. raimondii*	XP_012490256.1	7e-19
		Heat shock protein			
JK757122	543	hypothetical protein F383_34022	*G. arboreum*	KHG07754.1	2e-8
JK757125	727	Translation factor SUI1 homolog 2-like	*G. hirsutum*	XP_016728732.1	7e-74
JK757127	742	60S ribosomal protein L23-like	*G. hirsutum*	XP_016747847.1	9e-94
JK757130	497	FRIGIDA-like protein	*G. hirsutum*	XP_016731871.1	2e-26
JK757132	740	DNA methylation 1-like isoform X2	*G. hirsutum*	XP_016705962.1	5e-40
JK757132	375	Uncharacterized protein LOC105785704	*G. raimondii*	XP_012467285.1	9e-37
JK757154	336	Uncharacterized protein LOC107931081	*G. hirsutum*	XP_016718360.1	2e-15
JK757158	266	DNA polymerase delta subunit 4-like	*G. raimondii*	XP_012464216.1	2e-9
JK757160	760	Peroxidase P7-like isoform X2	*G. hirsutum*	XP_016724359.1	9e-66
JK757163	295	Translationally-controlled tumor protein	*G. raimondii*	XP_012461134.1	7e-8
JK757169	692	homolog isoform X2	*G. raimondii*	XP_012461574.1	7e-75
		Glutathione S-transferase F9-like			
JK757179		DNA-3-methyladenine glycosylase 1-like	*G. hirsutum*	XP_016689519.1	7e-7
JK757183	335	isoform X2	*G. hirsutum*	XP_016696495.1	3e-4
		Uncharacterized protein LOC107912702			
JK757186	300	Potassium channel KAT1	*G. raimondii*	XP_012455538.1	8e-32
JK757189	423	Putative aldo-keto reductase 1	*G. arboreum*	KHG27361.1	8e-22
JK757194	431	Peptidyl-prolyl cis-trans isomerase CYP19-3 isoform X3	*G. raimondii*	XP_012464987.1	7e-64
JK757195	672	Ubiquitin carboxyl-terminal hydrolase 2-like	*G. hirsutum*	XP_016739633.1	1e-34
JK757196	468	Uncharacterized protein LOC105770440	*G. raimondii*	XP_012447097.1	4e-30
JK757198	739	Uncharacterized protein LOC105800056	*G. raimondii*	XP_012486436.1	5e-76
JK757199	469	Phosphatase	*G. arboreum*	XP_017605169.1	5e-8
JK757202	686	Phosphoglucomutase, cytoplasmic isoform	*G. raimondii*	XP_012467251.1	4e-38
JK757204	747	X1			
		Heat shock protein	*G. raimondii*	XP_012445867.1	3e-100
JK757207	472	Peroxisomal fatty acid beta-oxidation multifunctional AIM1-like	*G. hirsutum*	XP_016720425.1	8e-20
JK757212	435	Heavy metal-associated isoprenylated plant protein 3-like	*G. hirsutum*	XP_016669560.1	9e-16
JK757219	460	Translation initiation factor 1A-like	*G. hirsutum*	XP_016740250.1	8e-30
JK757227	292	B3 domain-containing protein	*G. hirsutum*	XP_016678084.1	6e-5
		Os01g0234100-like isoform X3			
JK757233	558	Serine/threonine-protein kinase	*G. arboreum*	KHG03295.1	1e-58

JK757239	445	Polyubiquitin	*G. barbadense*	AAP40646.1	7e-51
JK757242	283	Callose synthase 7-like	*G. hirsutum*	XP_016678563.1	9e-37
JK757280	687	Zinc finger protein	*G. arboreum*	KHG09507.1	2e-35
JK757284	647	Endoplasmic reticulum-Golgi intermediate compartment protein 3-like	*G. raimondii*	XP_012464567.1	9e-45
JK757286	611	E3 ubiquitin-protein ligase RNF170-like	*G. raimondii*	XP_012447413.1	3e-10
JK757287	209	Ycf2	*G. somalense*	YP_006503402.1	2e-4
JK757291	714	hypothetical protein	*G. arboreum*	KHG27945.1	4e-22
JK757296	334	Lysine histidine transporter	*G. arboreum*	KHG11676.1	8e-20
JK757297	698	hypothetical protein	*G. arboreum*	KHG03575.1	7e-28
JK757301	332	Junction mediating and regulatory protein	*G. arboreum*	KHG07338.1	3e-4
JK757302	421	Coatomer subunit delta-like	*G. arboreum*	XP_017620365.1	1e-33
JK757310	300	STRUBBELIG-receptor family 8 isoform X1	*G. raimondii*	XP_012450336.1	9e-4
JK757312	249	60S ribosomal protein L17-1-like	*G. raimondii*	XP_012487644.1	7e-11
JK757316	677	Uncharacterized protein LOC107953297	*G. hirsutum*	XP_016744040.1	5e-40
JK757321	544	26S protease regulatory subunit 7-like	*G. raimondii*	XP_012454429.1	1e-51
JK757325	258	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 -like protein	*G. arboreum*	KHF98980.1	3e-11
JK757329	614	CSC1-like protein HYP1	*G. raimondii*	XP_012460084.1	5e-44
JK757336	440	Uncharacterized protein LOC105761689	*G. raimondii*	XP_012435028.1	5e-29
JK757341	600	hypothetical protein	*G. arboreum*	KHG07502.1	7e-21
JK757346	313	beta carbonic anhydrase 5, chloroplastic-like isoform X1	*G. raimondii*	XP_012445691.1	7e-27
JK757352	675	GATA transcription factor 24-like isoform X2	*G. hirsutum*	XP_016730602.1	4e-77
JK757357	340	Uncharacterized vacuolar membrane protein YML018C-like	*G. hirsutum*	XP_016710570.1	8e-34
JK757358	655	RNA-binding protein EIF1AD	*G. raimondii*	XP_012456238.1	5e-97
JK757360	352	Proline iminopeptidase-like	*G. hirsutum*	XP_016734470.1	3e-12
JK757361	664	Calcyclin-binding protein-like	*G. raimondii*	XP_012454629.1	5e-91
JK757362	572	Glutathione S-transferase DHAR2-like	*G. raimondii*	XP_012455168.1	7e-59
JK757367	526	Proteasome subunit alpha type-2-A-like	*G. raimondii*	XP_012454310.1	8e-90
JK757371	570	Auxin transport	*G. arboreum*	KHG12514.1	6e-43
JK757374	483	Transcription factor DIVARICATA-like	*G. hirsutum*	XP_016666455.1	8e-14
JK757374	401	Prefoldin subunit 4 isoform X2	*G. hirsutum*	XP_016725187.1	3e-25
JK757384	374	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase	*G. arboreum*	XP_017649018.1	6e-33
JK757398	446	Uncharacterized protein LOC105799707	*G. raimondii*	XP_012485884.1	8e-29
JK757402	395	Ubiquitin-like protein	*G. raimondii*	XP_012476412.1	8e-27
JK757412	304	Histone	*G. arboreum*	KHG21902.1	3e-30
JK757419	281	Uncharacterized protein LOC105800880	*G. raimondii*	XP_012487705.1	7e-18
JK757424	411	40S ribosomal protein S8	*G. hirsutum*	XP_016720360.1	3e-37
JK757435	650	26S protease regulatory subunit 7-like	*G. hirsutum*	XP_016688359.1	3e-37
JK757449	598	Gibberellin 2-beta-dioxygenase 1-like isoform X2	*G. hirsutum*	XP_016724318.1	9e-40
JK757467	460	Metallothionein	*G. hirsutum*	AAW47577.1	2e-31
JK757481	726	Uncharacterized protein At3g06530 isoform X3	*G. arboreum*	XP_017604320.1	1e-23
JK757484	673	ATP-dependent zinc metalloprotease FTSH 3, mitochondrial-like	*G. hirsutum*	XP_016683336.1	5e-85
JK757486	380	Oxygen regulatory nreC	*G. arboreum*	KHF97595.1	4e-14
JK757487	691	40S ribosomal protein S19-3-like	*G. hirsutum*	XP_016692083.1	7e-96
JK757503	422	Uncharacterized protein LOC105769242	*G. raimondii*	XP_012445185.1	4e-17
JK757507	369	CBL-interacting protein kinase 2-like	*G. hirsutum*	XP_016724097.1	7e-9
JK757510	271	Calcium-binding protein CML27	*G. hirsutum*	XP_016691150.1	8e-8
JK757532	349	Heat shock protein	*G. hirsutum*	ABW89470.1	3e-18
JK757538	197	Lysine-specific demethylase	*G. raimondii*	XP_012468557.1	2e-10
JK757549	436	Phosphoenolpyruvate carboxykinase [ATP] -like protein	*G. arboreum*	KHG26562.1	1e-28
JK757551	740	Glyceraldehyde-3-phosphate dehydrogenase	*G. arboreum*	KHG20184.1	3e-23
JK757555	251	Uncharacterized protein LOC105781470	*G. raimondii*	XP_012461467.1	2e-4

JK757559	769	Uncharacterized protein LOC108452640	*G. arboreum*	XP_017605930.1	3e-105
JK757560	687	Translational activator	*G. arboreum*	KHG25727.1	2e-29
JK757567	411	Wound-induced basic protein-like	*G. raimondii*	XP_012485360.1	2e-18
JK757585	790	Endochitinase	*G. arboreum*	KHF98356.1	4e-55
JK757587	692	Glutathione S-transferase F9-like	*G. hirsutum*	XP_016710368.1	5e-90
JK757596	453	Universal stress protein YxiE-like	*G. hirsutum*	XP_016680525.1	2e-40
JK757611	543	Uncharacterized protein LOC107940166 isoform X1	*G. hirsutum*	XP_016729086.1	2e-21
JK757628	349	uncharacterized protein LOC107916934	*G. hirsutum*	XP_016701797.1	8e-4
JK757641	414	Uncharacterized protein LOC107916578	*G. hirsutum*	XP_016701345.1	3e-7
JK757663	290	haloacid dehalogenase-like hydrolase domain-containing protein Sgpp isoform X2	*G. raimondii*	XP_012475757.1	5e-25
JK757665	549	Lipid-transfer protein DIR1	*G. hirsutum*	XP_01667	4e-61
JK757669	270	Signal peptide peptidase-like 4 isoform X4	*G. raimondii*	XP_012489957.1	2e-13
JK757695	498	Uncharacterized protein LOC105795069	*G. raimondii*	XP_012479982.1	6e-43
JK757698	325	Pathogenesis-related protein STH-2-like	*G. raimondii*	XP_012457235.1	1e-14
JK757703	346	3-deoxy-arabino heptulosonate 7-phosphate synthase	*G. hirsutum*	ABU43075.1	7e-14
JK757705	332	Uncharacterized protein LOC105771969	*G. raimondii*	XP_012448793.1	9e-11
JK757710	390	Elongation factor 1-alpha-like	*G. hirsutum*	XP_016722058.1	5e-39
JK757711	477	Metallothionein-like protein	*G. hirsutum*	AAV74186.1	7e-30
JK757715	606	Oxygen regulatory nreC	*G. arboreum*	KHF97595.1	5e-81
JK757720	697	Metallothionein-like protein 2	*G. hirsutum*	XP_016749146.1	7e-30

Table 3. Gene ID's along with KEGG orthologs and their associated pathways

Gene ID (GenBank Acc)	Species	KEGG Orthology	Role	Associated Pathway(s)
Gh_D12G1971 (JK757113)	*G. hirsutum*	K13993	HSP20 family protein	Genetic Information Processing Folding, sorting and degradation Protein processing in endoplasmic reticulum (ko04141)
Gorai.005G148100 (JK757118)	*G. raimondii*	K09487	heat shock protein	Protein processing in ER Plant-pathogen interaction (ko04626)
Gh_A11G2533 (JK757125)	*G. hirsutum*	K05286	Phosphatidylinositol glycan	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563) Metabolic pathways (ko01100)
Gh_D01G0610 (JK757127)	*G. hirsutum*	K02894	Large subunit ribosomal protein L23e	Genetic Information Processing Ribosome (ko03010)
Gh_A02G0834 (JK757132)	*G. hirsutum*	K11643	Chromodomain-helicase- DNA-binding protein 4	Enzymes (ko01000)
Gorai.007G246800 (JK757158)	*G. raimondii*	K03505	DNA polymerase delta Subunit	Purine metabolism (ko00230) Pyrimidine metabolism (ko00240)
Gh_A10G0565 (JK757160)	*G. hirsutum*	K00430	Peroxidase	Metabolic pathways (ko011000 Phenylpropanoid biosynthesis (ko00940) Metabolic pathways (ko01100) Biosynthesis of secondary metabolites (ko01110)
Gh_A05G2208 (JK757179)	*G. hirsutum*	K01247	DNA-3-methyladenine glycosylase II	Base excision repair (ko03410)
Gorai.013G120200 (JK757194)	*G. raimondii*	K03767	Peptidyl-prolyl cis-trans isomerase A (cyclophilin A)	Cellular Processes Cell growth and death (k01000) Chaperones and folding catalysts Protein folding catalysts (ko03110)
Gorai.002G176200 (JK757202)	*G. raimondii*	K01835	Phosphoglucomutase	Glycolysis / Gluconeogenesis (ko00010) Pentose phosphate pathway (ko00030) Galactose metabolism (ko00052) Fatty acid degradation (ko00071)
Gh_A06G0549 (JK757207)	*G. hirsutum*	K10527	Enoyl-CoA hydratase/3-hydroxyacyl-CoA	alpha-Linolenic acid metabolism (ko00592)

			Dehydrogenase	Metabolic pathways (ko01100) Biosynthesis of secondary metabolites (ko01110)
Gh_D07G1907 (JK757219)	*G. hirsutum*	K03236	Translation initiation factor 1A	RNA transport (ko03013)
GOBAR_AA01294 (JK757239)	*G. barbadense*	K08770	Ubiquitin C	PPAR signaling pathway (ko03320)
Gh_A04G1282 (JK757240)	*G. hirsutum*	K00706	1,3-beta-glucan synthase	Starch and sucrose metabolism (ko00500)
Cotton_A_25070 (JK757301)	*G. arboreum*	K10882	Crossover junction endonuclease EME1	Homologous recombination (ko03440)
Gorai.004G276000 (JK757312)	*G. raimondii*	K02880	Large subunit ribosomal protein L17e	Ribosome (ko03010)
Gorai.009G148400 (JK757321)	*G. raimondii*	K03061	26S proteasome regulatory subunit T1	Proteasome (ko03050)
Cotton_A_27223 (JK757325)	*G. arboreum*	K03965	NADH dehydrogenase (ubiquinone)	Oxidative phosphorylation (ko00190) Metabolic pathways (ko01100)
Gh_D12G0388 (JK757360)	*G. hirsutum*	K01259	Carbonic anhydrase	Nitrogen metabolism (ko00910)
Gorai.009G154900 (JK757361)	*G. raimondii*	K04507	Proline iminopeptidase	Arginine and proline metabolism (ko00330)
Gorai.009G152300 (JK757367)	*G. raimondii*	K02726	Calcyclin binding protein	Wnt signaling pathway (ko04310)
Cotton_A_22138 (JK757371)	*G. arboreum*	K13946	20S proteasome subunit alpha 2	Proteasome (ko03050)
Cotton_A_24443 (JK757384)	*G. arboreum*	K09667	Auxin influx carrier (AUX1 LAX family)	Plant hormone signal transduction (ko04075)
Cotton_A_22550 (JK757412)	*G. arboreum*	K11251	Protein O-GlcNAc transferase	Other types of O-glycan biosynthesis (ko00514)
Gh_A13G1144 (JK757424)	*G. hirsutum*	K02995	Histone H2A	Necroptosis (ko04217)
Gh_D05G1363 (JK757435)	*G. hirsutum*	K03061	Small subunit ribosomal protein S8e	Ribosome (ko03010)
Gh_A13G1308 (JK757449)	*G. hirsutum*	K04125	26S proteasome regulatory subunit T1	Proteasome (ko03050)
Cotton_A_03602 (JK757481)	*G. arboreum*	K14550	Gibberellin 2-oxidase	Diterpenoid biosynthesis (ko00904)
Gh_A07G1588 (JK757484)	*G. hirsutum*	K03798	U3 small nucleolar RNA- associated protein	Ribosome biogenesis in eukaryotes (ko03008)
Gh_D08G2451 (JK757487)	*G. hirsutum*	K02966	Cell division protease	Enzymes (ko01000), Metallo Peptidases (ko01002)
Gh_A11G0032 (JK757510)	*G. hirsutum*	K02183	Calmodulin	Ras signaling pathway (ko04014) Rap1 signaling pathway (ko04015) MAPK signaling pathway – plant (ko04016) Calcium signaling pathway (ko04020) cGMP-PKG signaling pathway (ko04022) cAMP signaling pathway (ko04024) Phosphatidylinositol signaling system (ko04070)
Gh_D11G1496 (JK757587)	*G. hirsutum*	K00799	Glutathione S-transferase	Glutathione metabolism (ko00480)

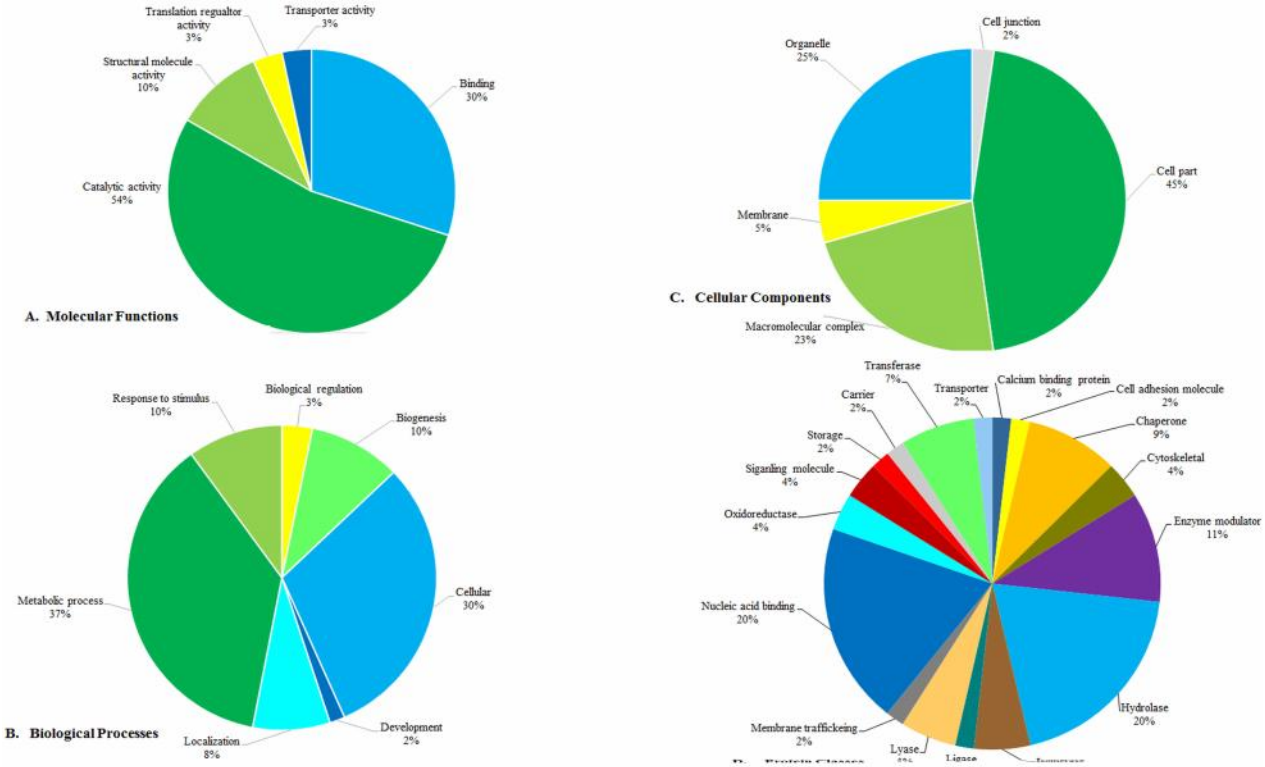

Fig 1. Functional annotation of drought stress responsive transcripts in cotton roots from cDNA library. Classification of ESTs based on (A) Cellular components, (B) Biological Processes, (C) Molecular Functions, (D) Protein classes

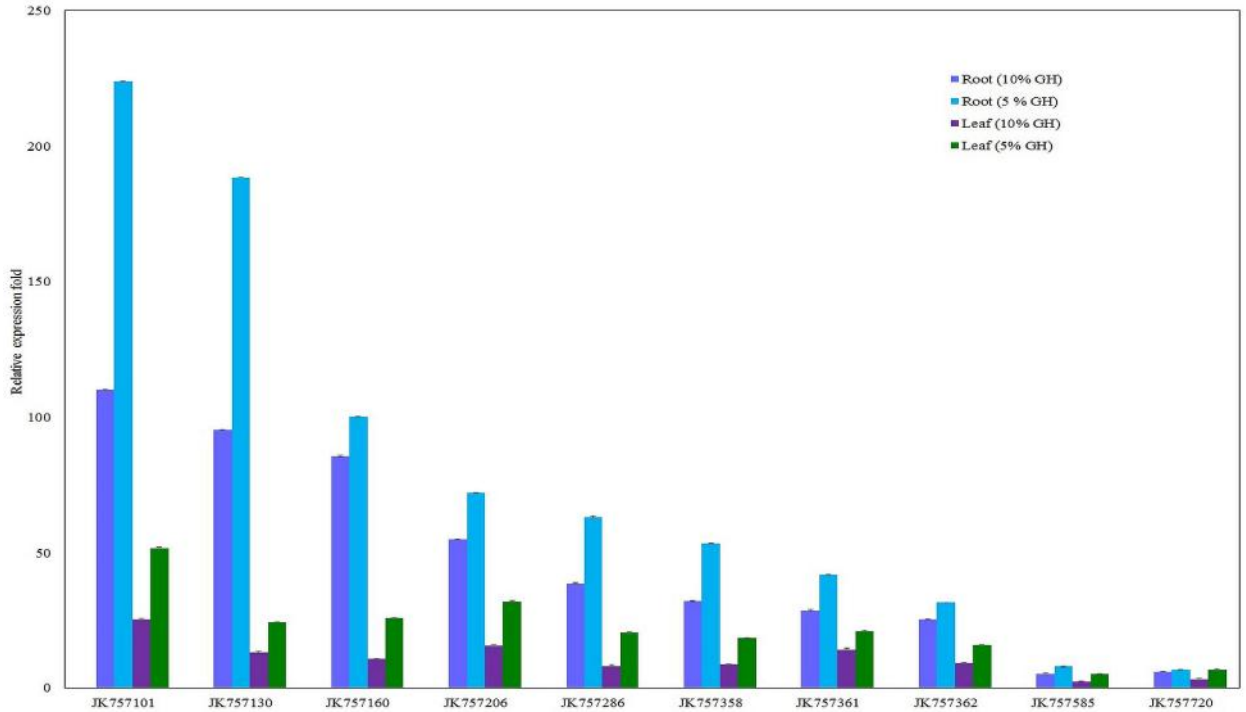

Fig 2. Relative expression of different unigenes in cotton roots as revealed by RT-qPCR.; JK757101 *WD Repeat*, JK757130-*FRIGIDA*, JK757160-*Peroxidase*, JK757206-*U Box*, JK757286-*E3 Ligase*, JK757358-*RNA binding*, JK757361-*Calcyclin*, JK757362-*Gluathione S-transferase*, JK757585-*Endochitinase*, JK757720-*Metallothionein.*

DISCUSSION

Many agro-physiological parameters related to drought tolerance have been established, RNA content, Relative water content (RWC) with decrease in water supply (Deblonde *et al*., 1999). The RWC is an important index in plants to measure plant water status, imitating its metabolic activity in tissues for dehydration tolerance (Anjum *et al*., 2011). Pronounced decline in RWC in plants leaves was observed with increasing water deficit in our study. Earlier studies also report decline in relative water contents with increase in water deprivation (Kumar *et al*., 2011; Meher *et al*., 2018). Relatively higher RWC observed in progressive mild stress than severe stress indicates that plants have the ability to sustain their water content under mild stress, whereas this ability lost under severe stress treatment in case of our findings. Alterations in RWC may be ascribed as ability of the variation to absorb more water and/or the ability to control water loss through stomata under osmotic stress (Bayoumi *et al*., 2008).

It is crucial to identify the differentially regulated genes and thorough understanding of stress tolerance at molecular and cellular levels (Ghorbel and Murphy, 2011). The crops whose complete genome sequence is not yet available, researchers have to opt the way by studying model genomes to explore EST sequences (Ewing *et al*. 1999). cDNA library constructed in our study reconnoitered drought responsive ESTs that can help in better understanding the molecular basis of drought tolerance in cotton. Overwhelming evidences also highlighted the role of stress genes and functionally efficient proteins involvement in biological processes, molecular functions and cellular structures in leaves and root tissues (Jiaa *et al*., 2015; Zhang *et al*., 2017).

Earlier cDNA libraries have been constructed for cotton related to drought responsive genes in leaves and roots (Zhang *et al*., 2009; Ranjan and Sawant, 2015). Still there is lack of molecular information regarding cotton root responses to drought stress. In this study, cDNA library containing 711 clones was constructed. Of these 711, 82 ESTs with an E value $1e^{-33}$ had significant homology / similarity to reported genes in database. While remaining (22 ESTs) with uncharacterized function to any genes in Genbank databases suggesting that these uncharacterized unigenes probably embroil in abiotic stress tolerance mechanism. These unknown genes are of particular interest and can be explored further for their role, sequence and structure at protein level. In our study, though a large number of sequences were potential drought responsive transcripts. These genes were involved in the catalytic, structural molecule, cellular, metabolic, nucleic acid binding, transporter and hydrolase activities assuming their role in drought induction. This implies that we successfully constructed cDNA library and have identified cotton root drought responsive genes.

The potential drought stress homologs engaged in biological processes reported in our study reveal their direct and / or indirect involvement in multiple stresses. Endochitinase (JK757105, JK757585) also known as EP3 chitinase has chitin binding activity and performs active biological functions in defense response, plant-type hypersensitive response, response to bacterium, wounding, somatic embryogenesis. Endochitinase being as type of pathogenesis related proteins are prompted under drought stress (Wang *et al*., 2016). JK757160 is peroxidase homolog (POX) P7 like protein involved in oxidative stresses. Different isoforms of POX are triggered by environmental stresses besides its involvement in plant growth and development. Various isoforms of POX are expressed under abiotic and biotic stresses (Li *et al*., 2009; Chiang *et al*., 2015). The homolog to the potassium channel (KAT) identified in this study was JK757186. KAT1 is the member of shaker family potassium ion channel located as integral membrane component and its active involvement in potassium ion transmembrane transport, membrane potential regulation and stomatal movement. Charged K^+ ion channels are involved in the regulation of guard cell volume. The cytosolic phosphoglucomutase (PGM) (JK757202) identified in roots cDNA library expresses during plant growth and developmental stages. PGM being as proteins of carbon/nitrogen metabolism was reported to be more abundant in plant roots under drought stress (Mohammadi *et al*., 2012a; Mohammadi *et al*., 2012b). JK757242 in our study shows close similarity with callose synthase central role in plant development and multiple abiotic stresses. Previous findings also identified the accumulation of callose in plasma membrane in plant tissues to various biotic (wound) stresses (Chen and Kim, 2009).

In our study, few unigenes have key biological role of proteasome-mediated ubiquitin-dependent protein catabolic process. 26S protease regulatory subunit (JK757321, JK757367 and JK757435) is proteasome complex with ATPase, hydrolase and peptidyl-prolyl cis-trans isomerase activity. 26S proteasome required for ubiquitin-dependent degradation, plant development and stress responses (Kurepa *et al*., 2009). Previous report illustrate that reduced 26S proteasome biogenesis results into increased hypersensitivity of heat shock while increases 26S proteasome biogenesis leads to boost cell capacity to destroy oxidized proteins which ultimately leads enhance stress tolerance during oxidation (Kurepa *et al*., 2009).

Metallothionein (MT) (JK757467, JK757711 and JK757720) have been reported in root elongation inhibition (Zhigang *et al*., 2006), biotic stress resistance (Wong *et al*., 2004) and abiotic / metal stress tolerance (Kholodova *et al*., 2010). In current study, JK757596

identified close homolog of universal stress protein (USP). USP improves the cell survival rate in response to stress when exposed to longer period of times, and may accomplish plants with broader spectrum of stress tolerance (Raphael *et al*., 2011). Previous findings suggest dignified involvement of USP to abiotic stresses like oxidative, salt, heat and drought (Zahur *et al*., 2009).

Our study enclosed few unigenes JK757125 JK757219 and JK757358 imparting their role as translation initiation factors (eIF). Previous studies reported TaeIF an overexpression in response to mild osmotic stress (Singh *et al*., 2007), elevated expression in roots (Yang *et al*., 2017), increased oxidases activities by enhancing protein synthesis and augmentation of ROS scavenging (Wang *et al*., 2012). EST JK757130 encodes FRIGIDA (FRI), a transcription factor functions in dehydration avoidance strategy (Lovell *et al*., 2013; Schmalenbach *et al*., 2014). Few unigenes JK757127, JK757312, JK757424 and JK757487 reported in current study are homologs to 60S and 40S ribosomal protein, involved in nucleic acid binding, translation and expressed during different growth and developmental plant stages. Environmental stresses regulate the ribosomal proteins and their overexpression in plants but still their abiotic stress mechanism is not very well understood (Xu *et al*.,2013; Liu *et al*., 2014). Ubiquitin carboxyl terminal based homologs JK757195 and JK757402 are engaged in hydrolase activity. The ubiquitin/26S proteasome proteolytic pathway plays an important role in development, stress responses and environmental adaptation by degrading short-lived and abnormal proteins (Hershko and Ciechanover, 1998; Callis and Vierstra, 2000). Among the unigenes involved in molecular processes, JK757233 serine threonine kinsase (SnRK) based homolog is involved in plant response to abiotic stresses, abscisic acid (ABA)-dependent plant development (Afzal *et al*., 2008) and metabolic signalling (Halford *et al*., 2003).

WD repeat (JK757101) identified in this study is expressed in different plant tissues during different plant developmental stages. WD repeats proteins are key players in abiotic stresses (Lee *et al*., 2010). Calcineurin binding protein- interacting protein kinases (CBL- CIPK) (JK757507, JK757510) perform key role in Ca^{2+} signals perception besides plant development (Eckert *et al*., 2014). Overexpression of CBL-CIPK confers drought tolerance in plants through the regulation of stomatal movement (Wang *et al*., 2016). Coatomer (JK757302) is clathrin adaptor complexes medium subunit family protein located in golgi, cytosol and regulate bodies development in endosperm under drought stress (Chen *et al*., 2017).

cDNA library showed that 21.15% ESTs had no homology to any protein in the NCBI database. These uncharacterized ESTs may provide novel and putative candidate genes for investigation to elucidate their role in drought stress. Transcriptome studies in past unveiled the uncharacterized transcription factors (Kumar *et al*.,2015), hypothetical proteins (Ding *et al*., 2014) and uncharacterized potential sequences (Govind *et al*., 2009) that modulate the drought tolerance. Furthermore, the identification of uncharacterized genes as stress responsive provides a function to these genes that could not be identified under non-stressed conditions.

Cellular, biological and molecular responses of plants to these stresses have been studied intensively (Hasegawa *et al*., 2000; Xiong *et al*., 2002). In our studies, we found different categories of genes had variable and differential expression in both roots and leaves tissues. Few unigenes affianced in biological processes showed an over expression under osmotic stress. We confirm the presence of cotton peroxidases (*POX;* JK757160) supposedly involved in abiotic stress responses of roots and leaves with a real time quantitative PCR (RT-qPCR) in root and investigate their role in under osmotic stress as reported earlier (Csiszár *et al*., 2012) suggesting that this transcripts may be components of the antioxidative defense mechanism activated especially in the drought tolerant cultivar. Another key unigene involved in biological function reported as E3 ubiquitin protein (*E3LIG*; JK757286) ligase found to upregulated in both roots and leaves tissues under stress that is supposed to have its functions in the drought stress response via the ABA-signaling pathway. E3 ubiquitin ligases been reported to be involved in ubiquitination-mediated degradation via the 26S proteasome by regulating ABA receptors degradation (Li *et al*., 2016). Previous reports suggested an overexpression of E3LIG based regulatory components in response to drought stress in plants (Kim *et al*., 2014).

Gluathione S transferase (*GST*) gene expression patterns to abiotic stresses demonstrated in plant systems explicate their role in enhancing stresses tolerance (Edwards and Dixon, 2005). A homolog (*GST*; JK757362) reported in this study displayed elevated expression pattern both in roots and leaves emphasizing its role in plant acclimation towards drought tolerance. Previous findings revealed the upregulated expression of *GST* to multiple abiotic stresses in above ground and underground plant tissues (Diao *et al*.,2010; Ding *et al*., 2017). Our results are in accordance with previous findings assuming responsiveness of *GST* to abiotic stresses.Current study entails homolog Endochitinase (*ECHT;* JK757585) explicating its inducible expression to osmotic stress emphasizing role in plants defense mechanism (Chen *et al*. 1994). Elevated *ECHT* expression have been reported in underground and above ground plant parts to multiple abiotic stresses (Behringer *et al*., 2015) and induced due to soil borne fungi and confers to biotic resistance and stress (Wu *et al*., 2012). Our findings indicate that enhanced expression of the *ECHT* could be responsible for the increased drought

tolerance as supported by earlier reports Furthermore; metallothionein (*MTT)* based homolog JK757720 showed variable expression under osmotic stress as in plants. *MTT* gene family play distinct and overlapping biological processes by the regulation of gene expression or signalling networks. Higher expression was of MTT was reported in current study and this overexpression of MTT gene family results in higher tolerance against abiotic stresses in plants due to scavenging of ROS production (Xue *et al*., 2009).

In the second category, few transcripts involved in molecular functions assessed by real time PCR exhibited an elevated expression in cotton roots and leaves tissues. Many RBPs have been shown their involvements in abiotic stresses (Ambrosone *et al*., 2012; Jung *et al*., 2013). Increased mRNA expression of *RBP* (JK757358) in different tissues is due to active participation of *RBP* ABA-dependent mechanisms of response to salt and drought stress (Ambrosone *et al*., 2015). Another molecular function based homolog FRIGIDA (JK757130) was up-regulated expounding its role under water deficit stress. Previous studies also reported overexpression of FRIGIDA suggesting that it enhances drought tolerance accumulating proline during water stress (Chen *et al*., 2018).

U-box domain containing protein (*U-BOX* JK757206), a class of E3 ubiquitin ligases exhibited an elevated expression in our experiment as its attributed towards U box role during ubiquitination, a cellular process plays an important role in the perception and signal transduction of hormone and various stress responses in higher plants (Hellmann and Estelle, 2002; Xu *et al*., 2015). Studies conducted earlier also reported strong up-regulation of *UBOX* genes in the roots under drought and salt stress (Cho *et al*., 2008). Our findings are best supported by previous studies signifying *UBOX* overexpression and enhanced drought tolerance (Liu *et al*., 2011). Expression of Calcyclin binding protein (*CBP*; JK757361), an Arabidopsis homologue of SIP (SIAH-interacting protein) was elevated in roots and leaves tissues in our studies. Increased CBP level may be due to the rapid generation of ROS primarily superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) and production of oxidative brust as well (Grant and Loake, 2000). Reports discussed earlier mentioned the highest expression of SIP in roots, root hairs and root tips and relatively low level in leaves, which is consistent with our studies (Kim *et al*., 2006).

In third category of expressed genes, *WD (JK757101)* was up-regulated in both roots and leaves compared to the control plants, which indicate the role of this gene during water deprivation. Increased expression of WD in plant roots with involvement in nodule formation, cell wall formation (Guerriero *et al*., 2015), response to hormones and abiotic stresses is well understood which supports our findings (Chuang *et al*., 2015).

Conclusion: We successfully constructed cDNA library from cotton roots and several potential transcripts encoding drought related proteins homology were identified. These unigenes were involved in multiple biological processes and different molecular processes under osmotic stress. Differential regulation of few drought responsive genes was validated through real time quantitative PCR. Several novel transcripts with no known functions may reveal their involvement in drought tolerance and these needs to be explore further. These genes with unknown functions need further exploration of novel mechanism that may be dynamic in cotton.

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REFERENCES

Afzal, A. J., A. J. Wood, and D.A. Lightfoot (2008). Plant receptor-like serine threonine kinases: roles in signaling and plant defense. Mol. Plant. Microbe-Interact. 21(5):507-517.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215(3):403-410.

Ambrosone, A., A. Costa, A. Leone, and S. Grillo (2012). Beyond transcription: RNA-binding proteins as emerging regulators of plant response to environmental constraints. Plant. Sci. 182:12-18.

Ambrosone, A., G. Batelli, R. Nurcato, V. Aurilia, P. Punzo, D. K. Bangarusamy, I. Ruberti, M. Sassi, A. Leone, A. Costa, and S. Grillo (2015). The Arabidopsis RNA-binding protein AtRGGA regulates tolerance to salt and drought Stress. Plant. Physiol. 168:292-306.

Anjum, S. A., X. Xie, L. C. Wang, M. F. Saleem, C. Man, and W. Lei (2011). Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6: 2026-2032.

Ashraf, J., D. Zuo, Q. Wang, W. Malik, Y. Zhang, M. A. Abid, H. Cheng, Q. Yang, and G. Song (2018). Recent insights into cotton functional genomics: progress and future perspectives. Plant. Biotech. J. 16, 699-713.

Bayoumi, T.Y., M. H. Eid, and E. M. Metwali (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotech. 7(14):2341-2352.

Barrs, H. D, and P. E Weatherly (1962). A re-examination of relative turgidity for estimating water deficits in leaves. Aus. J. Biol. Sci. 15:413-428.

Barozai, M.Y., and T. Husnain (2012). Identification of biotic and abiotic stress up-regulated ESTs in *Gossypium arboreum*. Mol. Biol. Rep. 39(2):1011-1018.

Behringer, D., H. Zimmermann, B. Ziegenhagen, and S. Liepelt (2015). Differential gene expression reveals candidate genes for drought stress response in *Abies alba* (Pinaceae). PloS. ONE 10(4): e0124564.

Bowman, M.J., W. Park, P.J. Bauer, J.A. Udall, J.T. Page, J. Raney, B.E. Scheffler. D. C. Jones, B. T. Campbell (2013). RNA-Seq transcriptome profiling of upland cotton (*Gossypium hirsutum* L.) root tissue under water-deficit stress. PLoS ONE. 8: e82634.

Callis, J. and R. D. Vierstra (2000). Protein degradation in signaling. Curr. Opin. Plant. Biol 3(5):381-386.

Chaves, M. M. and M. M. Oliveira (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot. 55:2365-2384.

Chen, Q., Y. Zheng, L. Luo, Y. Yang, X. Hu, and X. Kong (2018). Functional FRIGIDA allele enhances drought tolerance by regulating the P5CS1 pathway in *Arabidopsis thaliana*. Biochem. Biophys. Res. Commun. 495(1): 1102-1107.

Chen, R. D., L. X. Yu, A. F. Greer, H. Cheriti, and Z. Tabaeizadeh (1994). Isolation of an osmotic stress-induced and abscisic-acid-induced gene encoding an acidic endochitinase from *lycopersicon chilense*. Mol.Gen.Genet. 245(2):195-202.

Chen, X.Y. and J. Y. Kim (2009). Callose synthesis in higher plants. Plant. Signal. Behav. 4(6):489-492.

Chen, X.Y., Y. Yang, L. P. Ran, Z. D. Dong, E. J. Zhang, X. R. Yu, and F. Xiong (2017). Novel insights into miRNA regulation of storage protein biosynthesis during wheat caryopsis development under drought Stress. Front. Plant. Sci. 8:1707.

Chiang, C. M., L.F.O Chen, S.W Shih, and K.H Lin (2015). Expression of eggplant ascorbate peroxidase increases the tolerance of transgenic rice plants to flooding stress. J. Plant. Biochem. Biotech. 24(3):257-267.

Cho, S.K., M.Y. Ryu, C. Song, J.M. Kwak, and W.T. Kim (2008). Arabidopsis PUB22 and PUB23 are homologous U-Box E3 ubiquitin ligases that play combinatory roles in response to drought stress. Plant. Cell. 20(7):1899-1914.

Chuang, H.W., J.H. Feng, Y.L. Feng, and M.J. Wei (2015). An Arabidopsis WDR protein coordinates cellular networks involved in light, stress response and hormone signals. Plant. Sci. 241:23-31.

Csiszár, J., A. Gallé, E. Horváth, P. Dancsó, M. Gombos, Z. Váry, L. Erdei, J. Györgyey, and I. Tari (2012). Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress. Plant. Physiol. Biochem. 52:119-129.

Davies, W.J, and J.H. Zhang (1991). Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 42: 55-76.

Deblonde, P.M.K., A.J. Haverkort, and J.F. Ledent (1999). Responses of early and late potato cultivars to moderate drought conditions: Agronomic parameters and carbon isotope discrimination. Eur. J. Agron. 11(2):91-105.

Diao, G., Y. Wang, and C. Yang (2010). Functional characterization of a gluthathione S transferase gene from *Limonium bicolor* in response to several abiotic stresses. African. J. Biotech. 9(32):5060-5065.

Ding, H., Z.M. Zhang, F.F. Qin, L.X. Dai, C.J. Li, D.W. Ci, and W.W. Song (2014). Isolation and characterization of drought-responsive genes from peanut roots by suppression subtractive hybridization. Electronic. J. Biotech. 17(6):304-310.

Ding, N., A. Wang, X. Zhang, Y. Wu, R. Wang, H. Cui, R. Huang, and Y. Luo (2017). Identification and analysis of glutathione S-transferase gene family in sweet potato reveal divergent GST-mediated networks in aboveground and underground tissues in response to abiotic stresses. BMC. Plant. Biol. 17:225.

Dossa, K., M. A. Mmadi, R. Zhou, T. Zhang, R. Su, Y. Zhang, L. Wang, J. You, and X. Zhang (2019). Depicting the core transcriptome modulating multiple abiotic stresses responses in sesame (*Sesamum indicum* L.). Int. J. Mol. Sci. 20: 3930.

Eckert, C., J. N. Offenborn, T. Heinz, T. Armarego-Marriott, S. Schültke, C. Zhang, S. Hillmer, M. Heilmann, K. Schumacher, R. Bock, and I. Heilmann (2014). The vacuolar calcium sensors

CBL2 and CBL3 affect seed size and embryonic development in *Arabidopsis thaliana*. Plant. J 78: 146-156.

Edgerton, M.D (2009). Increasing crop productivity to meet global needs for feed, food, and fuel. Plant. Physiol. 149(1):7-13.

Edwards, R., and D.P. Dixon (2005). Plant glutathione transferases.Methods. Enzymol. 401:169-186.

Ewing, R.M., A.B. Kahla, O. Poirot, F. Lopez, S. Audic, and J.M. Claverie.(1999). Large-scale statistical analyses of rice ESTs reveal correlated patterns of gene expression. Genome. Res. 9(10):950-959.

Ghorbel, M.T, and D. Murphy (2011). Suppression subtractive hybridization. In: Adalberto, Merighi, editors. Neuropeptides, Methods and Protocols. Springer Protocols pp.237-259.

Govind, G., V.T. Harshavardhan, J.K. Patricia, R. Dhanalakshmi, M. K. Senthil, N. Sreenivasulu, and Udayakumar (2009). Identification and functional validation of a unique set of drought induced genes preferentially expressed in response to gradual water stress in peanut. Mol. Genet. Genomics. 281:591-605.

Grant, J.J., and G.J. Loake (2000). Role of ROIs and cognate redox signaling in disease resistance. Plant. Physiol. 124:21-29.

Graya, S.B., and S.M. Brady (2016). Plant developmental responses to climate change. Dev. Biol. 419(1):64-77.

Grayson, M. (2013). Agriculture and drought. Nature. 501 S1 10.1038/501S1a.

Guerriero, G., J.F. Hausman, and I. Ezcurra (2015). WD40-repeat proteins in plant cell wall formation: current evidence and research prospects. Front. Plant. Sci. 6:1112. doi: 10.3389/fpls.2015.01112.

Halford, N.G., S. Hey, D. Jhurreea, S. Laurie, R.S. McKibbin, M. Paul, and Y. Zhang (2003). Metabolic signaling and carbon partitioning: role of Snf1-related (SnRK1) protein kinase. J. Exp. Bot. 54:467-475.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu, and H.J. Bohnert (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 51:463-499.

Hellmann, H., and M. Estelle (2002). Plant development: regulation by protein degradation. Sci. 297(5582):793-797.

Hershko, A., and A. Ciechanover (1998). The ubiquitin system. Annu. Rev. Biochem. 67:425-479.

Isokpehi, R. D., S. S. Simmons, H. H.P. Cohly, S. I. N. Ekunwe, G. B. Begonia., and W. K. Ayensu (2011). Identification of drought-responsive universal stress proteins in viridiplantae. Bioinform. Biol Insights. 5: 41-58.

Jaakola ,L., A.M. irtPtilä, M. Halonen, A. Hohtola (2001). Isolation of high quality RNA from Bilberry (*Vaccinum myrtillus* L.) fruit. Mol. Biotechnol. 19:201-203.

Jamal, A., M.N. Shahid, B. Aftab, B. Rashid, M.B. Sarwar, B.B. Mohamed, S. Hassan, and T. Husnain (2014). Water stress mediated changes in morphology and physiology of *Gossypium arboreum* (Var FDH-786). J. Plant. Sci. 2:179-186.

Jiaa, D., B. Zhang, P.P. Zhang, J.Y. Zhang, Y.H. Liu, J.S. Wang, R.Y. Maa (2015). Identification of differentially expressed genes in *Alternanthera philoxeroides* under drought stress using suppression subtractive hybridization. Russian. J. Plant. Physiol. 62:93-100.

Jia, W., and J. Zhang (2008). Stomatal movements and long-distance signaling in plants. Plant. Signal. Behav. 3:772-777.

Jung, H.J., M.K. Kim, and H. Kang (2013). An ABA-regulated putative RNA binding protein affects seed germination of Arabidopsis under ABA or abiotic stress conditions. J. Plant. Physiol. 170:179-184.

Kholodova, V., K. Volkov, and V. Kuznetsov (2010). Plants under heavy metal stress in saline environments, In: I. Sherameti, A. Varma, editors. Soil heavy metals, Soil Biology. 19. Springer Berlin Heidelberg, pp. 163-183.

Kim, H., K. Lee, H. Hwang, N. Bhatnagar, D.Y. Kim, I.S. Yoon, M.O. Byun, S.T. Kim, K.H. Jung, and B.G. Kim (2014). Overexpression of PYL5 in rice enhances drought tolerance, inhibits growth, and modulates gene expression. J. Exp. Biol. 65:453-464.

Kim, Y.S., B.K. Ham, K.H. Paek, C.M. Park, and N.H. Chua (2006). An Arabidopsis homologue of human seven-in-absentia interacting protein is involved in pathogen resistance. Mol. Cells. 21(3):389-394.

Kumar, R.R., K. Karajol, and G.R. Naik (2011). Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (*Cajanus cajan* L. Millsp.). Recent. Res. Sci. Technol. 3(1):148-152.

Kumar, R.R., S. Yadav, D. Shrinivas, A.K. Srivastava, V. Shitole, and G.R. Naik (2015). Transcriptome of Pigeonpea roots under water deficit analyzed by suppression subtractive hybridization. J. Agr. Sci. Tech. 17:1333-1345.

Kurepa, J., S. Wang, Y. Li, and J. Smalle (2009). Proteasome regulation, plant growth and stress tolerance. Plant. Signal. Behav. 4(10):924-927.

Lawlor, D.W (2013). Genetic engineering to improve plant performance under drought: physiological

evaluation of achievements, limitations, and possibilities. J. Exp. Bot. 64(1):83-108.

Lee, S., J. Lee, K.H. Paek S.Y. Kwon, H. S. Cho, S. J. Kim, and J. M. Park (2010). A novel WD40 protein, BnSWD1, is involved in salt stress in *Brassica napus*. Plant. Biotech. Rep. 4(2):165-72.

Li, S., H. Chen, Z. Hou, Y. Li, C. Yang, D. Wang, C.P. Song (2019). Screening of abiotic stress-responsive cotton genes using a cotton full-length cDNA overexpressing Arabidopsis library. J. Integr. Plant. Biol. doi: 10.1111/jipb.12861.

Liu, Y.C., Y.R. Wu, X.H. Huang, J. Sun, and Q. Xie (2011). AtPUB19, a U-box E3 ubiquitin ligase, negatively regulates abscisic acid and drought responses in *Arabidopsis thaliana*. Mol. Plant. 4(6):938-946.

Li, Y.J., R.L. Hai, X.H. Du, X.N. Jiang, and H. Lu (2009). Over-expression of a Populus peroxisomal ascorbate peroxidase (PpAPX) gene in tobacco plants enhances stress tolerance. Plant. Breed. 128(4):404-410.

Li, M., Y. Li, J. Zhao, H. Liu, S. Jia, , J. Li, H. Zhao, S. Han, Y. Wang (2016). GpDSR7, a Novel E3 ubiquitin ligase gene in *Grimmia pilifera* is involved in tolerance to drought stress in Arabidopsis. PLoS ONE 11:e0155455.

Lim, C.W., C. Park, J.H. Kim, H. Joo, E. Hong, and S.C. Lee (2017). Pepper CaREL1, a ubiquitin E3 ligase, regulates drought tolerance via the ABA-signalling pathway. Sci. Rep. 7:477.

Liu, X.D., L. Xie, Y. Wei, X. Zhou, B. Jia, and J. Liu (2014). Abiotic stress resistance, a novel moonlighting function of ribosomal protein RPL44 in the halophilic fungus *Aspergillus glaucus*. Appl. Environ. Microbiol. 80(14):4294-4300.

Livak, K.J., and T.D. Schmittgen (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25(4):402-408.

Lovell, J.T., T.E. Juenger, S.D. Michaels, J.R. Lasky, A. Platt, J.H. Richards, X. Yu, H.M. Easlon, S. Sen, J.K. McKay (2013). Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation. Proc. Biol. Sci. 280(1763):20131043. doi: 10.1098/rspb.2013.1043.

Maqbool, A., M. Zahur, M. Irfan, U. Qaiser, B. Rashid, T. Husnain, and S. Riazuddin (2007). Identification, characterization and expression of drought related alpha-crystalline heat shock protein gene (GHSP) from desi cotton (*Gossypium arboreum* L.). Crop. Sci. 47:2437-2444.

Maqbool, A., M. Zahur, M. Irfan, M. Y. Barozai, B. Rashid, T. Husnain, and S. Riazuddin (2008). Identification and expression of six drought responsive transcripts through differential display in desi cotton (*Gossypium arboreum*). Mol. Biol. 42: 559-565.

Meherk., P. Shivakrishna, K.A. Reddy, and D.M. Rao (2018). Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi. J. Biol. Sci. 25(2):285-289.

Mohammadi, P.P., A. Moieni, S. Hiraga, and S. Komatsu (2012a). Organ-specific proteomic analysis of drought-stressed soybean seedlings. J. Proteomics. 75(6):1906-1923.

Mohammadi, P.P., A. Moieni, S. Komatsu (2012b). Comparative proteome analysis of drought sensitive and drought tolerant rapeseed roots and their hybrid F_1 line under drought stress. Amino. Acids. 43(5):2137-2152.

OECD-FAO (2019). OECD-FAO Agricultural Outlook 2019-2028, OECD Publishing, Paris/Food and Agriculture Organization of the United Nations, Rome. https://doi.org/10.1787/agr_outlook-2019-en.

Ogata, H., S. Goto, K. Sato, W .Fujibuchi, H. Bono, and M. Kanehisa (1999) KEGG: kyoto encyclopedia of genes and genomes. Nucleic. Acids. Res. 27(1):29-34.

Parida, A.K., V.S. Dagaonkar, M.S. Phalak, G. Umalkar, and L.P. Aurangabadkar (2007). Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant. Biotechnol. Rep. 1(1):37-48.

Park, B.J., Z. Liu, A. Kanno, and T. Kameya (2005). Increased tolerance to salt and water deficit stress in transgenic lettuce (*Lactuca sativa* L.) by constitutive expression of LEA. Plant. Growth. Regul. 45(2):165-171.

Ramachandra, R.A., K.V. Chaitanya, and M. Vivekanandan (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant. Physiol. 161(11):1189-1202.

Ranjan, A., and S. Sawant (2015). Genome-wide transcriptomic comparison of cotton (*Gossypium herbaceum*) leaf and root under drought stress. 3. Biotech. 5(4): 585-596.

Riaz, M., J. Farooq, G. Sakhawat, A. Mahmood, M. Sadiq, and M. Yaseen (2013). Genotypic variability for root/shoot parameters under water stress in some advanced lines of cotton (*Gossypium hirsutum* L.). Genet. Mol. Res. 12(1): 552-561.

Sambrook, J., E.F. Fritsch, and T. Maniatis (1998). Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, 1998 ed Cold Spring Harbor - New York.

Schmalenbach, I., L. Zhang, M. Reymond, J.M. Jiménez-Gómez (2014). The relationship between flowering time and growth responses to drought in the Arabidopsis Landsberg erecta × Antwerp-1 population. Front. Plant. Sci. 5:609. doi: 10.3389/fpls.2014.00609.

Shahid, M.N., A. Jamal, B. Rashid, B. Aftab, and T. Husnain (2012). Identification and isolation of salt-stress responsive transcripts from *Gossypium arboreum* L. Turk. J. Biol. 36: 746-756.

Singh, G., M. Jain, R. Kulshreshtha, J.P. Khurana, S. Kumar, and P. Singh (2007). Expression analysis of genes encoding translation initiation factor 3 subunit g (TaeIF3g) and vesicle-associated membrane protein-associated protein (TaVAP) in drought tolerant and susceptible cultivars of wheat. Plant. Sci. 173(6):660-669.

Statista. (2019) https://www.statista.com/ statistics /263055/cotton-production-worldwide-by-top-countries/.

Umezawa, T., M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki, and K. Shinozaki (2006). Engineering drought tolerance in plants: discovering and tailoring genes unlock the future. Curr. Opinion. Biotech. 17(2):113-122.

Wang, J., X. Mao, R. Wang, A. Li, G. Zhao, J. Zhao, and R. Jing (2019). Identification of wheat stress-responding genes and TaPR-1-1 function by screening a cDNA yeast library prepared following abiotic stress. Sci. Rep. 9:141.

Wang, L., C. Xu, C. Wang, and Y. Wang (2012). Characterization of a eukaryotic translation initiation factor 5A homolog from *Tamarix androssowii* involved in plant abiotic stress tolerance. BMC. Plant. Biol. 12:118.

Wang, W., B. Vinocur, and A. Altman (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 218(1):1-14.

Wang, Y., F. Liu, Y. Ren, Y. Wang, X. Liu, W. Long, D. Wang, J. Zhu, X. Zhu, R. Jing, M. Wu, Y. Hao, L. Jiang, C. Wang, H. Wang, Y. Bao, and J. Wan (2016). GOLGI TRANSPORT 1B regulates protein export from the endoplasmic reticulum in rice endosperm cells. Plant. Cell. 28(11): 2850-2865.

Wang, Y., T. Sun, L. Tingting, M. Wang, G. Yang, X., and He, G. Y. (2016). A CBL-interacting protein kinase TaCIPK2 confers drought tolerance in transgenic tobacco plants through regulating the stomatal movement. *PLoS ONE* 11:e0167962.

Wong, H.L., T. Sakamoto, T. Kawasaki, K. Umemura, and K. Shimamoto (2004). Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant. Physiol. 135(3):1447-1456.

Xiong, L., K.S. Schumaker, and J.K. Zhu (2002). Cell signaling during cold, drought, and salt stress. The. Plant. Cell. 14suppl: S165-S183.

Wu, B., B. Zhang, Y. Dai, L. Zhang, K. Shang-Guan, Y. Peng, Y. Zhou, and Z. Zhu (2012). *Brittle Culm15* encodes a membrane-associated chitinase-like protein required for cellulose biosynthesis in rice. Plant. Physiol. 159(4): 1440-1452.

Xu, J., X-J. Xing, Y-S. Tian, R-H. Peng, Y. Xue, W. Zhao, and Q-H. Yao (2015). Transgenic Arabidopsis plants expressing tomato glutathione s-transferase showed enhanced resistance to salt and drought stress. PLoS One. 10(9): e0136960.

Xue, T., X. Li, W. Zhu, C. Wu, G. Yang, and C. Zheng (2009). Cotton metallothionein GhMT3a, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. J. Exp. Bot. 60(1):339-349.

Xu, T., K. Lee, L. Gu, J.I. Kim, and H. Kang (2013). Functional characterization of a plastid-specific ribosomal protein PSRP2 in *Arabidopsis thaliana* under abiotic stress conditions. Plant. Physiol. Biochem. 73:405-411.

Yan, H., S. Kikuchi, P. Neumann, W. Zhang, Y. Wu, F. Chen, and J. Jiang (2010). Genome-wide mapping of cytosine methylation revealed dynamic DNA methylation pattern associated with genes and centromeres in rice. Plant. J. 63(3):353-365.

Yang, G., L. Yu, Y. Wang, C. Wang, and C. Gao (2017). The translation initiation factor 1A (*TheIF1A*) from *Tamarix hispida* is regulated by a Dof transcription factor and increased abiotic stress tolerance. Front. Plant. Sci. 8:513.

Zahur, M., A. Maqbool, M. Irfan, M.Y.K. Barozai, B. Rashid, and S. Riazuddin (2009). Isolation and functional analysis of cotton universal stress protein promoter in response to phytohormones and abiotic stresses. Mol. Biol. 43(4):578-585.

Zahur, M., A. Maqbool, M. Irfan, A. Jamal, M.N. Shahid, B. Aftab, T. Husnain (2012). Identification and characterization of a novel gene encoding myb-box binding zinc finger protein in *Gossypium arboreum*. Biologia. Plantarum. 56(4):641-647.

Zhang, L., F.G. Li, C.L. Liu, C.J. Zhang, and X.Y. Zhang (2009). Construction and analysis of cotton (*Gossypium arboreum* L.) drought-related cDNA library. BMC. Res. Notes. 2:120.

Zhang, F., G. Zhu, L. Du, X. Shang, C. Cheng, B. Yang, Y. Hu, C. Cai, and W. Guo (2016). Genetic regulation of salt stress tolerance revealed by RNA-Seq in cotton diploid wild species, *Gossypium davidsonii*. Sci. Rep. 6:20582.

Zhang, F., Y. Zhou, M. Zhang, X. Luo, and J. Xie (2017). Effects of drought stress on global gene expression profile in leaf and root samples of Dongxiang wild rice (*Oryza rufipogon*). Biosci. Rep. 37(3). pii: BSR20160509.

Zhigang, A., L. Cuijie, Z. Yuangang, D. Yejie, A. Wachter, R. Gromes, and T. Rausch (2006). Expression of BjMT2, a metallothionein 2 from *Brassica juncea*, increases copper and cadmium tolerance in *Escherichia coli* and *Arabidopsis thaliana*, but inhibits root elongation in *Arabidopsis thaliana* seedlings. J. Exp. Bot. 57:3575-3582

Zhou, B., L. Zhang, A. Ullah, X. Jin, X. Yang, X. Zhang (2016). Regulation of drought stress response multiple stress responsive genes by sequencing a normalized cDNA library from sea-land cotton (*Gossypium barbadense* L.). PLoS. ONE. 11(3):e0152927.